

Application No.: 09/258600
Group Art Unit: 1636
Docket No.: 50370-60637DIV (formerly CPI-012CP4DV)

Amendment to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-43: (Cancelled)

44. (Currently Amended) The yeast cell of claim [43]100 in which the peptide is an agonist for the surrogate receptor.

45. (Currently Amended) The yeast cell of claim [43]100 in which the peptide is an antagonist for the surrogate receptor.

46. (Currently Amended) The yeast cell of claim [43]100, which further comprises a G protein, said G protein comprising a $G\alpha$ subunit, wherein said $G\alpha$ subunit is chimeric.

47. (Currently Amended) The A yeast cell of claim 46 having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor;

(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell; and

(c) a G protein comprising a $G\alpha$ subunit, wherein said $G\alpha$ subunit is chimeric and wherein the amino terminal portion of the $G\alpha$ subunit is substantially homologous with the $G\alpha$ subunit of a yeast G protein and the remainder is substantially homologous with the corresponding portion of a $G\alpha$ subunit of a heterologous G protein; and said modulation is a selectable or screenable event.

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48. (Currently Amended) The yeast cell of claim [43]100 wherein said yeast cell comprises an the endogenous pheromone system protein, wherein said protein is not produced in functional form.
49. (Currently Amended) The yeast cell of claim [43]100 wherein the heterologous peptide is secreted by the cell into the periplasmic space, from which it interacts with said surrogate.
50. (Original) The yeast cell of claim 49, wherein the heterologous peptide is expressed in the form of a precursor peptide comprising a cleavable leader peptide and a mature peptide, which leader peptide directs secretion of said heterologous peptide.
51. (Original) The yeast cell of claim 50 wherein the leader peptide corresponds to a leader peptide of the *Saccharomyces cerevisiae* α factor or a-factor.
52. (Currently Amended) The yeast cell of claim [43]100 in which a wild-type pheromone of the yeast pheromone system is not secreted.
53. (Original) The yeast cell of claim 49 wherein the heterologous peptide is also expressed in a nonsecretory form.
54. (Currently Amended) The yeast cell of claim [43]100, wherein the cell is a mutant strain having a pheromone signal pathway that is desensitized at slower rate relative to the wild type strain under the same conditions of continuous stimulation of the pheromone signal pathway.
55. (Original) The yeast cell of claim 54 in which the endogenous *SST2* gene is not functionally expressed.

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56. (Currently Amended) The yeast cell of claim [43]100, in which the endogenous *FAR1* gene is not functionally expressed.

57. (Currently Amended) The yeast cell of claim [43]100, further comprising a selectable marker that is activated by the pheromone signal pathway.

58. (Original) The yeast cell of claim 57, said selectable marker comprising a pheromone-responsive promoter which is substantially homologous with an endogenous pheromone-responsive promoter, operably linked to a foreign selectable gene.

59. (Previously Presented) The yeast cell of claim 58 wherein the selectable gene is an *HIS3* gene.

60. (Original) The yeast cell of claim 58 wherein the homologous wild-type promoter is the *FUS1* promoter.

61. (Currently Amended) The yeast cell of claim [43]100 wherein the cells belong to the species *Saccharomyces cerevisiae*.

62. (Currently Amended) A yeast culture comprising a plurality of yeast cells according to claim [43]100, said yeast cells collectively expressing a peptide library.

63. (Currently Amended) A method of assaying a peptide for modulation of the activity of a non-yeast surrogate for a pheromone system protein which comprises providing yeast cells according to claim [43]100, which cells functionally express said heterologous surrogate and said heterologous peptide, and determining by detecting a change in said selectable or screenable event whether the pheromone signal pathway is activated or inhibited by the interaction of said surrogate and said peptide.

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64. (Original) The method of claim 63 in which the cells comprise a pheromone-responsive selectable marker, and cells are selected for expression of a peptide having the desired activating or inhibiting effect.

65. (Original) The method of claim 63 in which the cells comprise a pheromone-responsive screenable marker, and cells are screened for expression of a peptide having the desired activating or inhibiting effect.

66. (Original) A method of assaying a peptide library for activity of a non-yeast pheromone system protein surrogate which comprises providing a yeast culture according to claim 62, whose cells each functionally express said surrogate and a peptide of said library, said culture collectively expressing the entire peptide library, and determining whether the pheromone signal pathway is activated or inhibited by said peptides in each of the cells of said culture.

67. (Currently Amended) ~~The~~A yeast cell of claim 43 having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, wherein said surrogate is the C5a receptor and performs in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor; and

(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell; and said modulation is a selectable or screenable event.

68. (Currently Amended) ~~The~~A method of claim 64 assaying a peptide for modulation of the activity of a non-yeast surrogate for a pheromone system protein which comprises:

(a) providing yeast cells, wherein each of the yeast cells has a pheromone system and comprises:

(i) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, in which the surrogate is human Mdr1[,] and performs in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor;

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(ii) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell; and

(iii) a pheromone-responsive selectable marker; wherein said modulation is a selectable or screenable event; and

wherein the cells grow on histidine-free media only if the surrogate transports α -factor, the cells are galactose-sensitive only if the surrogate transports α -factor, and endogenous pleiotropic drug resistance genes have been inactivated;

(b) determining by detecting a change in said selectable or screenable event whether the pheromone signal pathway is activated or inhibited by the interaction; and

(c) selecting said cells selected for expression of a peptide having the desired activating or inhibiting effect.

69. (Currently Amended) A mixture of recombinant yeast cells, each cell of which comprises a yeast cell according to claim 100[:]

~~— (i) a pheromone system generating a detectable signal;~~

~~(ii) an expressible gene construct encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor; and~~

~~(iii) an expressible gene construct encoding a heterologous peptide, wherein collectively the mixture of cells expresses a library of said heterologous peptides, and modulation of the pheromone system by the heterologous peptide provides the detectable signal.~~

70. (Original) The recombinant cells of claim 69, wherein the yeast pheromone receptor is inactivated.

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71. (Original) The recombinant cells of claim 69, wherein each cell further comprises a marker gene construct containing a marker gene in operative linkage with one or more transcriptional regulatory elements responsive to the pheromone system, expression of the marker gene providing the detectable signal.

72. (Original) The recombinant cells of claim 71, wherein the marker gene that gives rise to a detectable signal selected from the group consisting of: β galactosidase, alkaline phosphatase, horseradish peroxidase, exoglucanase, luciferase, and chloramphenicol acetyl transferase.

73. (Previously Presented) The recombinant cells of claim 71, wherein the marker gene that gives rise to a detectable signal is a *III*S3 gene.

74. (Original) The recombinant cells of claim 69, wherein the population of heterologous peptides includes at least 10^3 different peptide sequences.

75. (Original) The recombinant cells of claim 69, wherein the population of heterologous peptides includes at least 10^7 different peptide sequences.

76. (Original) The recombinant cells of claim 69, wherein the yeast cell is a *Saccharomyces* cell.

77. (Currently Amended) A mixture of recombinant yeast cells, each cell of which comprises a yeast cell according to claim 100[:]

(i) a pheromone system generating a detectable signal;

(ii) an expressible gene construct encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor; and

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(iii) wherein said yeast cell further comprises an expressible gene construct encoding a heterologous peptide, said heterologous peptide including a signal sequence for secretion into the periplasmic space, wherein collectively the mixture of cells expresses a library of said heterologous peptides, and modulation of the pheromone system by the heterologous peptide provides the detectable signal.

78. (Original) The recombinant cells of claim 77, wherein the yeast pheromone receptor is inactivated.

79. (Original) The recombinant cells of claim 77, wherein each cell further comprises a marker gene construct containing a marker gene in operative linkage with one or more transcriptional regulatory elements responsive to the pheromone system, expression of the marker gene providing the detectable signal.

80. (Original) The recombinant cells of claim 79, wherein the marker gene encodes a gene product that gives rise to a detectable signal selected from the group consisting of: β -galactosidase, alkaline phosphatase, horseradish peroxidase, exo glucanase, luciferase, and chloramphenicol acetyl transferase.

81. (Previously Presented) The recombinant cells of claim 79, wherein the marker gene that gives rise to a detectable signal is a *HIS3* gene.

82. (Original) The recombinant cells of claim 77, wherein the population of heterologous peptides includes at least 10^3 different peptide sequences.

83. (Original) The recombinant cells of claim 77, wherein the population of heterologous peptides includes at least 10^7 different peptide sequences.

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84. (Original) The recombinant cells of claim 77, wherein the yeast cell is a *Saccharomyces* cell.

85. (Currently Amended) A method for identifying potential effectors of a yeast pheromone surrogate, comprising:

(i) providing a mixture of recombinant yeast cells, each cell of which comprises a yeast cell according to claim 100

(a) ~~a pheromone system generating a detectable signal;~~

(b) ~~an expressible gene construct encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor; and~~

(c) ~~an expressible gene construct encoding a heterologous peptide,~~

wherein collectively the mixture of cells expresses a library of said heterologous peptides, and modulation of the pheromone system by the heterologous peptide provides the detectable signal; and

(ii) isolating cells from the mixture which exhibit the detection signal.

86. (Original) The method of claim 85, wherein the yeast pheromone receptor is inactivated.

87. (Original) The method of claim 85, wherein said heterologous peptide includes a signal sequence for secretion into the periplasmic space.

88. (Original) The method of claim 85, wherein each cell of the mixture further comprises a marker gene construct containing a marker gene in operative linkage with one or more transcriptional regulatory elements responsive to the signal transduction activity of the cell surface receptor protein, and wherein expression of the marker gene provides the detection signal.

89. (Original) The method of claim 88, wherein the marker gene encodes a gene product that gives rise to a detection signal selected from the group consisting of: β galactosidase, alkaline

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phosphatase, horseradish peroxidase, exo glucanase, luciferase, and chloramphenicol acetyl transferase.

90. (Previously Presented) The method of claim 88, wherein the marker gene that gives rise to a detectable signal is a *HIS3* gene.

91. (Original) The method of claim 85, wherein the population of heterologous peptides includes at least 10^3 different peptide sequences.

92. (Original) The method of claim 85, wherein the population of heterologous peptides includes at least 10^7 different peptide sequences.

93. (Original) The method of claim 85, wherein the yeast cell is a *Saccharomyces* cell.

94. (Currently Amended) ~~The~~A yeast cell of claim 43, having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor; and

(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event; and wherein the yeast cell lacks ras function in the presence of cAMP.

95. (Original) The yeast cell of claim 94, wherein the yeast cell comprises a *cara* mutation.

96. (Currently Amended) The yeast cell of claim [43]100 wherein the yeast cell responds to a factor.

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97. (Previously Presented) The yeast cell of claim 96, wherein the yeast cell expresses Ste3p.

98. (Currently Amended) ~~The~~A yeast cell of claim 43, having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor; and
(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event; and wherein the yeast cell responds to a factor and fails to grow on galactose.

99. (Currently Amended) ~~The~~A yeast cell of claim 97, having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor;
(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell; and
(c) a mutated form of Gal7 or Gal10; wherein said modulation is a selectable or screenable event; and wherein the yeast cell responds to a factor, expresses Ste3p, and expresses Gal1 under the control of a pheromone responsive promoter ~~and further comprises a mutated form of Gal7 or Gal10.~~

100. (Previously Presented) A yeast cell having a pheromone system, which cell comprises:
(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, and

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(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event, and wherein said heterologous polypeptide is selected from the group consisting of agonists for the surrogate receptor and antagonists of the surrogate receptor.

101. (Previously Presented) A yeast cell having a pheromone system, which cell comprises:

(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor;

(b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event, and wherein said heterologous polypeptide is selected from the group consisting of agonists for the surrogate receptor and antagonists of the surrogate receptor; and

(c) chimeric $G\alpha$ subunit, wherein the amino terminal portion of the $G\alpha$ subunit is substantially homologous with the $G\alpha$ subunit of a yeast G protein and the remainder is substantially homologous with the corresponding portion of a $G\alpha$ subunit of a heterologous G protein.

102. (Currently Amended) [A] ~~The yeast cell having a pheromone system, which cell comprises:~~

~~(a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, and~~

~~(b) a second heterologous gene encoding a heterologous peptide of claim 100, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event, and wherein (i) said heterologous polypeptide is secreted by the cell into the~~

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~~periplasmic space, from which it interacts with said surrogate, or (ii)~~ is expressed in nonsecretory form.

Claims 103-108: (Cancelled)

109. (Previously Presented) A yeast cell having a pheromone system, which cell comprises
- (a) a first heterologous gene encoding a heterologous surrogate of a yeast pheromone receptor, said surrogate performing in the pheromone system of the yeast cell a function naturally performed by said yeast pheromone receptor, and
 - (b) a second heterologous gene encoding a heterologous peptide, wherein said heterologous peptide modulates the interaction of said surrogate with said pheromone system in the yeast cell, and said modulation is a selectable or screenable event, and wherein the heterologous peptide is 2 to 200 amino acids in length.